PERGAMON

Soil Biology and Biochemistry 31 (1999) 1083–1090

Soil Biology & Biochemistry

Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture

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Accepted 28 December 1998

Abstract

Potential C and N mineralization and soil microbial biomass C were determined following disturbance (i.e. drying and sieving) pretreatments in five soils varying in texture (30–350 mg clay g^{-1} soil) from the southern Piedmont USA. Soil disturbance by drying (i.e. rewetting following drying at 55°C for 72 h) of intact soil cores resulted in a flush of C mineralization (70% to 2.5-fold greater) during 0–3 d of incubation, but was not significantly different during 3–10 and 10–24 d periods compared with field-moist-intact soil cores. Soil disturbance by sieving resulted in greater C mineralization earlier than later in the incubation and led to significant immobilization of N of surface soil where respiration was highest. Increasing soil disturbance through smaller sieve openings resulted in a 10–60% greater flush of C mineralization that may have been due to disruption of macroaggregates, which protected soil organic C. With a conditioning period of 10 d following rewetting of dried soil, soil microbial biomass C was unaffected by drying or extent of sieving. Soil texture (i.e. clay content) did not interact with disturbance effects. Immobilization of N was predominant in surface soils (0–40 mm) of this bermudagrass pasture, where mineralizable C was very high. Carbon mineralization during 0–3 d was highly related ($r^2 = 0.96 \pm 0.04$) to C mineralization during 0–24 d, basal soil respiration and soil microbial biomass C, although increasing soil disturbance (i.e. drying and extent of sieving) altered these relationships in a predictable manner. I conclude that dried and coarsely sieved soil compares favorably to field-moist-intact soil cores for estimating soil microbial biomass and potential activity in landscapes scoured by various degrees of erosion. Published by Elsevier Science Ltd.

1. Introduction

Soil microbial biomass and potential activity are important components for understanding early changes in biological soil quality following a change in land management (Powlson et al., 1987). Use of intact-field-moist soil cores for determination of potential C and N mineralization and microbial biomass may better approximate in situ conditions (Cabrera and Kissel, 1988a,b), but use of disturbed soil (i.e. dried and sieved) (i) allows sampling at many locations to average across spatial variability, (ii) reduces number of samples to be analyzed without compromising representivity of a treatment or ecosystem, (iii) increases flexibility during initial processing of samples by avoiding refrigeration or immediate handling and the need to determine initial water content of each sample separately in order to adjust moisture content to an optimum and (iv) avoids potential seasonal complications of sampling when field-moist soil may be very dry or very wet.

Homogenization of soil by breaking soil aggregates is known to cause a flush of C mineralization that is likely due to release of organic matter protected within aggregates (Powlson, 1980; Elliott, 1986; Beare et al., 1994). Further, protection of soil organic matter from decomposition by microorganisms by the clay fraction has been observed (Crasswell and Waring, 1972; Merckx et al., 1985). Therefore, determination of potential C and N mineralization and microbial biomass

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0038-0717/99/$ - see front matter Published by Elsevier Science Ltd.
PII: S0038-0717(99)00022-X

may be biased when using dried and sieved soil compared with intact-field-moist soil cores in soils with varying clay content or aggregation, but this information is not known.

Soils of the Southern Piedmont USA have undergone severe erosion and degradation as a result of historically intensive tillage (Trimble, 1974). Uneroded soils in the region have a sandy A horizon and a distinct Bt horizon. Erosion has exposed higher clay content subsoil, resulting in typical landscapes with varying surface soil textures within hectares. When attempting to determine land management implications on biological soil quality (i.e. microbial biomass and potential activity) within a diverse textural landscape, questions arise as to the appropriate methodology for obtaining unbiased estimates.

My objectives were to (i) determine potential C and N mineralization and microbial biomass C of soils subjected to various disturbance regimes (i.e. moisture and sieve opening), (ii) distinguish between the disturbance effects of drying and extent of sieving on microbial biomass and potential activity and (iii) determine if low-activity clay content interacts with drying and sieving on microbial biomass and potential activity.

2. Materials and methods

A variably eroded, upland landscape near Farmington, GA (33°20′N, 83°23′W) dominated by Cecil–Madison–Pacolet soils (clayey, kaolinitic, thermic Typic Kanhapludults) had been conventionally cultivated with wheat (*Triticum aestivum* L.), soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) for several decades prior to establishment of coastal bermudagrass (*Cynodon dactylon* L.) in 1991. Bermudagrass received ≈ 200 kg N ha^{-1} yr^{-1} in split applications of NH_4NO_3 during spring and summer. Bermudagrass was grazed by yearling Angus steers from May until October to maintain 1.5–2.5 Mg available forage ha^{-1} beginning in 1994. Mean annual temperature is 16.5°C, mean annual precipitation is 1250 mm, and mean annual pan evaporation is 1560 mm.

Five locations within a 12-ha area were selected to represent a range of clay contents commonly found in the region. Clay content was determined from the Ap horizon (148 ± 94-mm depth) using the hydrometer method (Gee and Bauder, 1986), immediately prior to grass establishment in 1991 (Table 1). Twelve soil cores (41-mm dia) were collected to a depth of 80 mm within a 0.25-m^2 area at each of the five locations on 13 December 1996. Soil cores were divided into 0–40 and 40–80 mm sections and placed into 120 screw-top glass jars (43-mm dia, 40-mm height). Soil during sampling was near saturation (89 mm of rainfall occurred during the 2 weeks prior to sampling with 23 mm of that falling 2 days prior to sampling).

Of the 120 total cores collected, 20 cores were cooled to 4°C for 1 week until the start of the incubation, while 100 cores were oven-dried at 55°C for 3 d. Oven-drying was chosen as a standard procedure to facilitate rapid drying and to be consistent with previous experimental procedure (Franzluebbers and Arshad, 1997; Franzluebbers et al., 1998, 1999). Twenty of the dried cores remained intact and 80 cores were gently crushed to pass either a 7.9, 4.7, 2.0 or 0.5-mm screen and repacked into glass jars. Bulk density of repacked–dried–sieved soils was lower than that of intact-moist or intact-dried soils (Table 2).

Dried soil was moistened to 55% water-filled pore space at the start of an aerobic incubation and intact-field-moist soil remained at 67 ± 10% water-filled pore space at time of sampling. Soil was placed into a 1-l

Table 1
Characteristics of the five soils with different clay contents (values are mean ± standard deviation among six disturbance regimes)

Clay content (mg g^{-1})	Soil organic C (mg g^{-1})	Soil organic N (mg g^{-1})	Bulk density (Mg m^{-3})	Soil water (g g^{-1})
0–40 mm depth				
30	29 ± 7	2.0 ± 0.4	1.1 ± 0.1	0.37 ± 0.08
110	24 ± 3	1.7 ± 0.2	1.2 ± 0.1	0.37 ± 0.07
190	68 ± 17	5.4 ± 1.3	0.9 ± 0.1	0.57 ± 0.13
270	23 ± 6	1.8 ± 0.6	1.2 ± 0.1	0.39 ± 0.05
350	28 ± 4	2.1 ± 0.3	1.2 ± 0.1	0.39 ± 0.08
40–80 mm depth				
30	10 ± 1	0.7 ± 0.1	1.5 ± 0.1	0.25 ± 0.06
110	10 ± 1	0.7 ± 0.1	1.5 ± 0.1	0.26 ± 0.07
190	34 ± 12	2.6 ± 0.9	1.2 ± 0.1	0.40 ± 0.13
270	12 ± 1	0.9 ± 0.1	1.4 ± 0.1	0.33 ± 0.09
350	15 ± 3	1.1 ± 0.2	1.4 ± 0.1	0.32 ± 0.09

Bulk density was prior to sieving. Soil water content was during incubation.

Table 2
Soil bulk density during incubation as affected by disturbance regime and depth of sampling (values are mean ± standard deviation among five soils with varying clay content)

Disturbance regime (moisture and sieve opening)	Bulk density ($Mg\ m^{-3}$)	
	0–40 mm depth	40–80 mm depth
Moist, intact	1.05 ± 0.13	1.44 ± 0.09
Dried, intact	1.11 ± 0.09	1.41 ± 0.05
Dried, 7.9 mm	0.82 ± 0.08	0.98 ± 0.13
Dried, 4.7 mm	0.84 ± 0.12	0.97 ± 0.14
Dried, 2.0 mm	0.84 ± 0.12	0.95 ± 0.12
Dried, 0.5 mm	0.88 ± 0.07	1.00 ± 0.14

canning jar along with a vial containing 10 ml of 1 M NaOH to trap evolved CO_2 and a vial of water to maintain humidity. Canning jars were sealed and incubated in the dark at 25 ± 1°C for up to 24 d. Alkali traps were replaced at 3 and 10 d. Carbon mineralization during 0–3, 3–10 and 10–24 d periods was determined as total CO_2 evolved by titrating alkali to a phenolphthalein endpoint with 1 M HCl (Anderson, 1982). Duplicate jars of each soil textural class, depth and disturbance regime were maintained until 10 d. At the end of 10 d, one of the duplicate samples was fumigated with $CHCl_3$ (Jenkinson and Powlson, 1976). Following removal of vapors at the end of 24 h, soil was returned to the canning jar and incubated for 10 d under the same conditions as before. Soil microbial biomass C was calculated as the CO_2–C evolved from fumigated soil without subtraction of a control using an efficiency factor of 0.41 (Voroney and Paul, 1984).

Inorganic N (NO_3–N + NO_2–N and NH_4–N) was determined for each soil textural class, depth and disturbance regime at 0 and 24 d of incubation from dried soil (55°C, 48 h) sieved to < 2 mm (10 g soil, 20 ml of 2 M KCl) using Cd reduction and salicylate autoanalyzer techniques (Bundy and Meisinger, 1994). Potential N mineralization was calculated as the difference in inorganic N between 0 and 24 d.

Since the variation in soil organic C among soil cores within a depth and soil textural class was large (Table 1), potential C and N mineralization were expressed per unit of total C and N present within each core. Soil organic C and N were determined from a dried (55°C, 48 h) and ball-milled (5 min) subsample taken at the end of 24 d of incubation using dry combustion.

Data were subjected to analysis of variance using the general linear model procedure of SAS (SAS Institute Inc., 1990). The effect of clay content on soil biological properties was tested using linear regression. Three orthogonal contrasts were used to separate the disturbance effects of (i) drying (moist-intact vs. dried-intact), (ii) sieving (moist-intact + dried-intact vs. all dried and sieved) and (iii) extent of sieving (linear effect of four sieving sizes, which were log-transformed). Interaction of disturbance regime with clay content was tested with clay content as a covariate. Effects were considered significant at $P \leq 0.1$.

3. Results

Carbon mineralization per unit of soil organic C averaged across disturbance regimes decreased with increasing clay content (Fig. 1). This effect was significant during the 0–3, 3–10, 10–24 and 0–24 d incubation periods at both soil depths, except during 0–3 d of incubation at 40–80 mm depth. Net N mineralization and soil microbial biomass C, however, were not significantly affected by clay content.

The linear effect of clay content on C and N mineralization and soil microbial biomass did not interact significantly with disturbance regimes at either soil depth (data not shown). This indicates that any effect of soil disturbance (i.e. moisture and sieve opening) was independent of soil clay content.

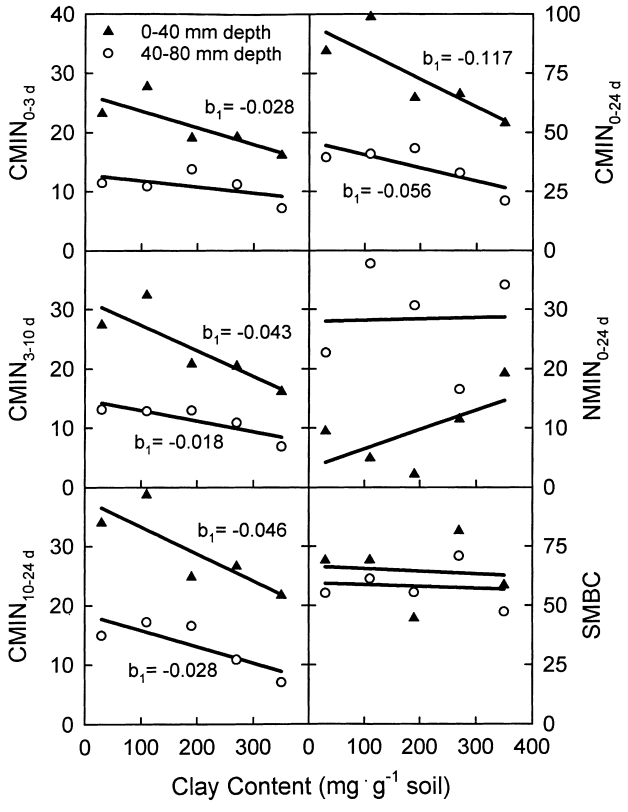

Fig. 1. Effect of clay content on C mineralization (CMIN, mg g^{-1} total C) during 0–3, 3–10, 10–24 and 0–24 d incubation periods, on net N mineralization (NMIN, mg g^{-1} total N) during 0–24 d incubation period, and on soil microbial biomass C (SMBC, mg g^{-1} total C) at soil depths of 0–40 and 40–80 mm. Significant regressions at $P \leq 0.1$ are labelled with a slope coefficient.

The disturbance effect of drying on C mineralization was significant only during 0–3 d of incubation at both soil depths (Fig. 2). Carbon mineralization from dried-intact soil compared with field-moist-intact soil was 2.58 ± 0.89-fold greater (mean difference $\pm$ standard deviation among five soil textural classes and two depths) during 0–3 d, 1.47 ± 0.56-fold greater during 3–10 d (data not shown) and 1.04 ± 0.23-fold greater during 10–24 d of incubation. Due to the large effect during 0–3 d of incubation, drying resulted in significantly greater C mineralization during 0–24 d of incubation at a depth of 0–40 mm (1.42 ± 0.45-fold), but not significantly at a depth of 40–80 mm (data not shown). There was no significant effect of drying on net N mineralization or soil microbial biomass C.

The disturbance effect of sieving was not consistent among biological properties. Soil disturbance by sieving compared with intact cores resulted in a significant increase in C mineralization during 0–3 d at both soil depths, in C mineralization during 0–24 d at a depth of 40–80 mm, and in soil microbial biomass C at a depth of 40–80 mm (Fig. 2). However, sieving also resulted in a significant decrease in C mineralization during 10–24 d at a depth of 0–40 mm and in net N mineralization at a depth of 0–40 mm. Reduced net N mineralization due to sieving probably occurred due to mixing of surface residues that resulted in greater N immobilization.

Increasing disturbance of soil by sieving through smaller openings resulted in an increase in C mineralization at a depth of 0–40 mm only during 0–3 d and not during other incubation periods (Fig. 2). However at a depth of 40–80 mm, C mineralization increased with increasing disturbance through smaller sieve openings during all incubation periods. Increasing disturbance through smaller sieve openings had no significant effect on net N mineralization or soil microbial biomass C.

Carbon mineralization during 0–3 d of incubation was highly related to C mineralization during 0–24 d of incubation, basal soil respiration (i.e. the linear rate

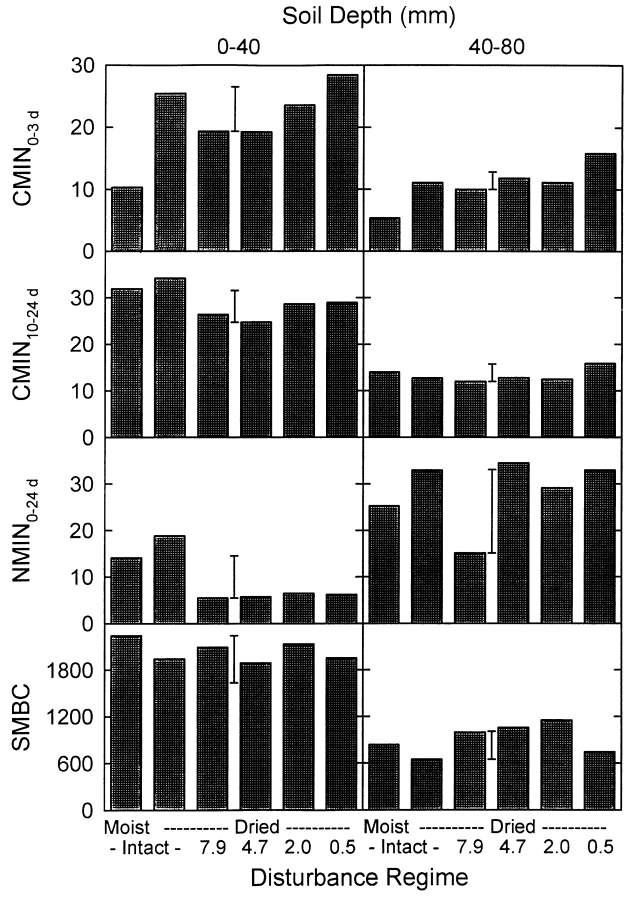

Fig. 2. Effect of disturbance regime (moisture-sieve opening: moist-intact, dried-intact, dried-7.9 mm, dried-4.7 mm, dried-2.0 mm and dried-0.5 mm) on C mineralization (CMIN, mg g^{-1} total C) during 0–3 and 10–24 d incubation periods, on net N mineralization (NMIN, mg g^{-1} total N) during 0–24 d incubation period and on soil microbial biomass C (SMBC, mg kg^{-1} soil) at soil depths of 0–40 and 40–80 mm. Error bars are $LSD_{P \leq 0.1}$.

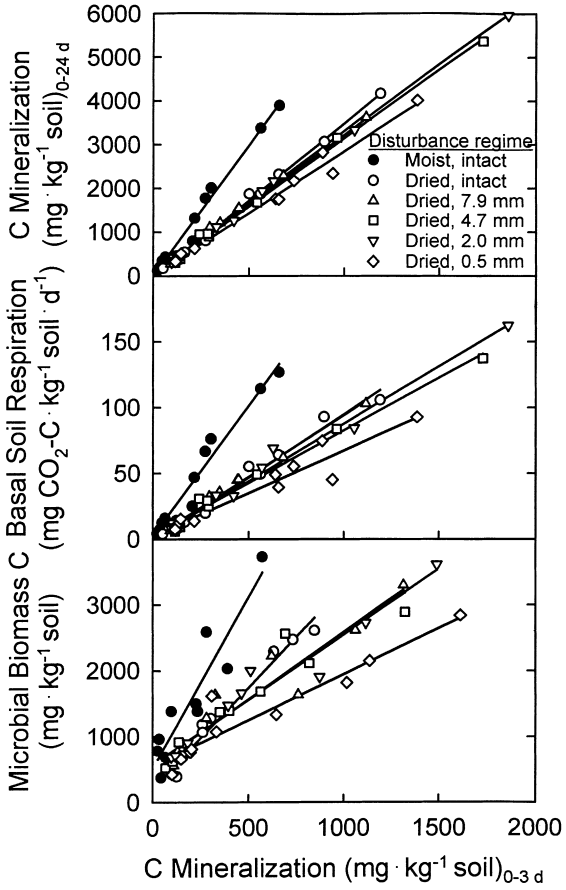

Fig. 3. Relationships of C mineralization during 0–24 d of incubation, basal soil respiration and soil microbial biomass C with C mineralization during 0–3 d of incubation as affected by disturbance regime (moisture and sieve opening). See Table 3 for coefficients of regression equations.

Table 3
Regression coefficients for relationships between C mineralization during 0–3 d of incubation ($CMIN_{0-3\ d}$, mg kg^{-1} soil) and C mineralization during 0–24 d ($CMIN_{0-24\ d}$, mg kg^{-1} soil), basal soil respiration (BSR, mg kg^{-1} soil d^{-1})$_{10-24\ d}$, soil microbial biomass C (SMBC, mg kg^{-1} soil) and net N mineralization during 0–24 d at a depth of 40–80 mm only ($NMIN_{0-24\ d}$, mg kg^{-1} soil) as affected by disturbance regime

	Disturbance regime (moisture and sieve opening)					
Regression parameter	moist intact	dried intact	dried 7.9 mm	dried 4.7 mm	dried 2.0 mm	dried 0.5 mm
$CMIN_{0-24\ d}$-to-$CMIN_{0-3\ d}$ ($n = 10$)						
β_0	-8 ± 86	-39 ± 42	30 ± 32	28 ± 41	-3 ± 39	25 ± 97
β_1	6.0 ± 0.3	3.5 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	2.8 ± 0.1
r^2	0.98	0.99	0.99	0.99	0.99	0.98
BSR-to-$CMIN_{0-3\ d}$ ($n = 10$)						
β_0	2 ± 4	-1 ± 3	1 ± 2	3 ± 2	0 ± 3	3 ± 5
β_1	0.20 ± 0.01	0.10 ± 0.01	0.09 ± 0.003	0.08 ± 0.003	0.09 ± 0.004	0.07 ± 0.01
r^2	0.96	0.98	0.99	0.99	0.98	0.91
SMBC-to-$CMIN_{0-3\ d}$ ($n = 10$)						
β_0	506 ± 191	210 ± 77	548 ± 144	566 ± 133	560 ± 115	551 ± 126
β_1	5.2 ± 0.7	3.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	1.4 ± 0.2
r^2	0.87	0.97	0.91	0.91	0.95	0.90
$NMIN_{0-24\ d}$-to-$CMIN_{0-3\ d}$ (40–80 *mm depth only*, $n = 5$)						
β_0	0 ± 8	9 ± 20	15 ± 9	12 ± 8	17 ± 24	14 ± 11
β_1	0.37 ± 0.08	0.18 ± 0.12	-0.01 ± 0.05	0.15 ± 0.03	0.12 ± 0.08	0.09 ± 0.02
r^2	0.88	0.43	0.00	0.90	0.42	0.81

of C mineralization during 10–24 d of incubation) and soil microbial biomass C (Fig. 3). The ratio of C mineralization during 0–24 d-to-C mineralization during 0–3 d decreased with increasing disturbance, i.e. by drying and by sieving (Table 3). Likewise, the ratios of basal soil respiration-to-C mineralization during 0–3 d and soil microbial biomass C-to-C mineralization during 0–3 d also decreased with increasing disturbance. For all three sets of regressions, the slope (i) decreased due to drying, (ii) decreased due to sieving and (iii) decreased with extent of sieving ($P < 0.001$), due to very strong relationships within a disturbance regime (i.e. moisture and sieve opening).

Net N mineralization was not related to C mineralization during 0–3 d at a depth of 0–40 mm (Fig. 4), but at a depth of 40–80 mm was highly related when soil was field-moist-intact and when soil was dried and sieved through 4.7-mm or 0.5-mm openings (Table 3). Substantial N immobilization likely occurred, especially at a depth of 0–40 mm, due to the very high respiration rates in these soils under pasture.

4. Discussion

Coefficient of variation in soil organic C and N among soil cores within individual 0.25-m^2 areas (samples separated by 0.1–0.7 m) was $19 \pm 10\%$. This variation was similar to the coefficient of variation among 159 samples collected at 30-m intervals within a 12-ha area (samples separated by 30–500 m) of the same pasture ($23 \pm 2\%$) (Franzluebbers, unpublished data). This suggests that in order to characterize the effect of landscape position or imposed management on soil organic matter content, numerous subsamples even on a very small scale need to be collected. If intact soil cores were used, several-fold greater number of separate analyses would be required to estimate the

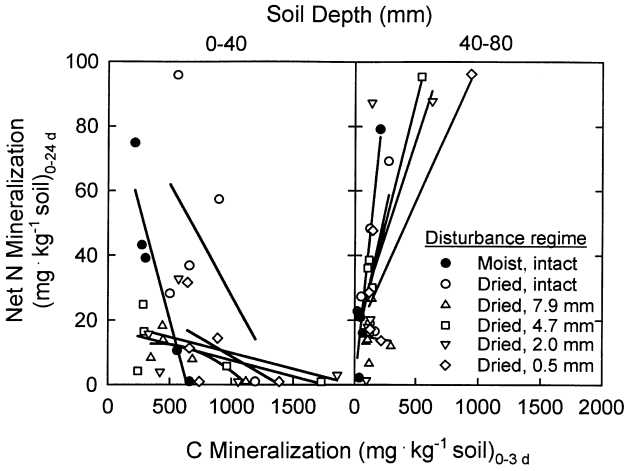

Fig. 4. Relationship of net N mineralization during 0–24 d of incubation with C mineralization during 0–3 d of incubation as affected by soil depth and disturbance regime (moisture and sieve opening). See Table 3 for coefficients of regression equations.

mean stock of soil organic C compared with a composite from numerous subsamples.

The effect of increasing clay content on C and N mineralization in this study adds to mixed results obtained previously. Carbon mineralization from added substrates has been shown to be more rapid in soils with low compared with high clay content (Merckx et al., 1985). During most incubation periods in the current study, this same effect of clay content on C mineralization as a portion of total C was observed, but not on N mineralization as a portion of total N. In contrast, N mineralization as a portion of total N in Dutch grassland soils decreased with increasing clay content, but C mineralization as a portion of total C was unaffected by clay content (Hassink, 1994). It is unclear why clay content influences C and N mineralization differently within the same soil. Both C and N mineralization as a portion of total organic matter were unaffected by clay content in soils from Texas (Franzluebbers et al., 1996a). Differences in response of C and N mineralization to clay content among the current and aforementioned studies may be partially due to the type of clay present. Clays in soils from the southeastern USA are dominated by kaolinite with low surface charge, while clays in soils from northern and western USA are generally dominated by montmorillonite with high surface charge. High surface-charged clays attract oppositely charged functional groups of various organic molecules that protect them from decomposition by microorganisms until physically disturbed (Crasswell and Waring, 1972).

Soil textural effects on C and N mineralization may also be influenced by the quality of soil organic matter. In the current study, soils with high clay content have recently had much of the historical surface soil eroded away exposing high clay-content subsoil with relatively little time for direct contact with fresh organic inputs. Soil organic C and N in subsoil would likely be more chemically stable because of the sparse availability of readily mineralizable substrates.

Physical protection of organic matter by clay has been proposed to explain observations of increasing soil organic matter and microbial biomass with increasing clay content (van Veen et al., 1984). It is experimentally difficult to clearly distinguish the direct effect of clay content on physical protection of soil organic matter from the indirect effects of associated changes in other biogeochemical properties, which are often influenced by clay content. However, physical protection of a potentially labile source of organic matter has been experimentally demonstrated by disrupting macroaggregates (Elliott, 1986; Gupta and Germida, 1988). Mineralization of substrates made available following crushing has been shown to be rapid, in which 28 and 86% of that released during 24 d of incubation occurred during the first 3 and 10 d, respectively (Franzluebbers and Arshad, 1997). Current results exhibited a similarly rapid response in C mineralization with increasing disturbance by extent of sieving (i.e. smaller openings). Carbon mineralization at a depth of 0–40 mm increased with extent of sieving during 0–3 d, but not later. At a depth of 40–80 mm, C mineralization increased with extent of sieving during all incubation periods, but the magnitude of difference decreased with time.

Current results indicated no interaction of clay content with disturbance by sieving (i.e. to destroy any potential macroaggregate-protected soil organic C and N) on microbial biomass or potential activity at either soil depth. This suggests that these soils had similar amounts of macroaggregate-protected soil organic C, irrespective of clay content. These results are in contrast with other studies conducted primarily with montmorillonite as the dominant clay type. Macroaggregate-protected soil organic C was found to increase with increasing clay content, at least at a depth of 0–50 mm (Franzluebbers and Arshad, 1997). Also in contrast to the current study, the portion of total N as mineralizable N was unaffected by clay content in dried and sieved soil, but decreased with increasing clay content in field-moist-intact soil (Cabrera and Kissel, 1988b). The difference in soil textural response of N mineralization to disturbance in the aforementioned study suggests more macroaggregate-protected soil organic N was released with drying and sieving with increasing clay content. Further, an increase in net N mineralization during 14 d of incubation occurred in soils sieved to <0.18 mm compared with <2 mm and the extent of this increase was positively related to the clay content (Crasswell and Waring, 1972). From the contrast in the results of the current study with those of previous studies, it appears that kaolinitic clays with low surface activity may not offer the same degree of physical protection to soil organic matter and microbial biomass that montmorillonitic clays with high surface activity provide. However, this contrast in macroaggregate-protected soil organic N due to clay type was not observed by Crasswell and Waring (1972).

Drying soil is known to cause a flush in C and N mineralization (Birch, 1958). The effect of drying on net N mineralization was shown to subside within the first 2–3 d of incubation (Cabrera, 1993). Franzluebbers et al. (1996b) observed that 90% of the flush in C mineralization due to drying occurred within 4–10 d. Current results corroborate this short-lived flush of activity due to drying. These observations suggest that near steady-state microbial activity (i.e. basal soil respiration) can be measured during the 10–24-d period of incubation. In support of this conclusion, basal soil respiration estimated using the linear rate of C mineralization during 10–24 d of incubation

was highly related ($r^2 = 0.99$, $n = 18$) and only 5% greater than using a nonlinear curve fitting procedure (Franzluebbers and Arshad, 1996). The effect of this finding is that, independent of clay content or soil depth, basal soil respiration can be estimated with dried–sieved soils following a 10-d conditioning period.

Carbon mineralization during 0–3 d of incubation was highly related to cumulative C mineralization during 24 d, basal soil respiration and soil microbial biomass C under all disturbance regimes, despite an additional flush of activity due to drying and sieving. Similar relationships between C mineralization during 1 d after rewetting of dried soil and microbial biomass and potential activity were reported by Franzluebbers et al. (1996b) for eight soils in Texas. It appears that the flush of activity following rewetting of dried soil is an essential part of the biologically available pool of organic matter composed of soil microbial biomass and basal soil respiration (Jenkinson, 1966; Kieft et al., 1987; van Gestel et al., 1991). Similar to the analysis of soils with predominantly montmorillonitic clays (Franzluebbers et al., 1996b), there was no effect of kaolinitic clay content on the relationships between C mineralization during 0–3 d and C mineralization during 0–24 d, basal soil respiration, soil microbial biomass C and net N mineralization during 0–24 d in the current study. It appears that a short-term C mineralization assay could offer an inexpensive, rapid, and reliable estimate of microbial biomass and potential activity.

I conclude that dried and coarsely sieved soil compares favorably to field-moist-intact soil cores for estimating soil microbial biomass and potential activity. Further, no evidence was found to suggest that drying and sieving soil would bias estimates of microbial biomass and potential activity due to increasing kaolinitic clay content, which was hypothesized to harbor more protected soil organic matter.

Acknowledgements

I am grateful to Mr. A. David Lovell for processing samples and to Dr. R. Rus Bruce, Dr. Jean L. Steiner, Dr. John A. Stuedemann and Dr. Stan R. Wilkinson for establishing and maintaining this pasture experiment.

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